Autophagy and cancer – issues we need to digest

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Summary

Autophagy is an evolutionarily conserved catabolic pathway that has multiple roles in carcinogenesis and cancer therapy. It can inhibit the initiation of tumorigenesis through limiting cytoplasmic damage, genomic instability and inflammation, and the loss of certain autophagy genes can lead to cancer. Conversely, autophagy can also assist cells in dealing with stressful metabolic environments, thereby promoting cancer cell survival. In fact, some cancers rely on autophagy to survive and progress. Furthermore, tumour cells can exploit autophagy to cope with the cytotoxicity of certain anticancer drugs. By contrast, it appears that certain therapeutics require autophagy for the effective killing of cancer cells. Despite these dichotomies, it is clear that autophagy has an important, if complex, role in cancer. This is further exemplified by the fact that autophagy is connected with major cancer networks, including those driven by p53, mammalian target of rapamycin (mTOR), RAS and glutamine metabolism. In this Commentary, we highlight recent advances in our understanding of the role that autophagy has in cancer and discuss current strategies for targeting autophagy for therapeutic gain.

This article is part of a Minifocus on Autophagy. For further reading, please see related articles: 'Ubiquitin-like proteins and autophagy at a glance' by Tomer Shpilka et al. (*J. Cell Sci.* **125**, 2343-2348) and 'Autophagy and cell growth – the yin and yang of nutrient responses' by Thomas Neufeld (*J. Cell Sci.* **125**, 2359-2368).

Key words: Autophagy, Cancer, Therapy

Introduction

Autophagy is a catabolic process that targets cellular organelles and cytoplasmic constituents to the lysosomes for degradation (Mizushima et al., 2008). There are three main types of autophagy, namely macroautophagy, microautophagy and chaperone-mediated autophagy, which are characterised by different mechanisms of delivering cargoes to the lysosome (Mizushima et al., 2008). Macroautophagy is often simply (and hereafter) referred to as autophagy. It is the best-characterised autophagic process and is the focus of this Commentary.

Autophagy is characterised by the formation of double-membrane vesicles – termed autophagosomes – that sequester cargo that is destined for degradation in the lysosome (Eskelinen and Saftig, 2009; Mizushima et al., 2008). Autophagy is constitutively active at basal levels in most, if not all, cells. The level of autophagic activity as well as the types of cargo can, however, be modulated in response to a variety of intracellular and extracellular cues, such as those encountered in disease states, including starvation, hypoxia and other forms of metabolic stress (Wilkinson and Ryan, 2010). Under nutrient-replete conditions, autophagy is thought to selectively degrade long-lived proteins and organelles as a means of recycling cell contents and maintaining cellular homeostasis and integrity. The destiny of autophagic cargo, even following degradation, can also be modulated. Under most circumstances, constituent parts of digested cytoplasmic material are recycled into biosynthetic pathways. However, under conditions of metabolic stress, the products of autophagy can be further catabolised to fuel ATP synthesis (Lum et al., 2005).

Owing to the multiple roles carried out by autophagy to maintain cellular viability and fidelity, it does not come as a surprise that autophagy has been implicated in protecting organisms against a variety of diseases (Cadwell et al., 2008; Kroemer and White, 2010; Mathew et al., 2007a; Rosenfeldt and Ryan, 2011). In this Commentary, we will focus on the role that autophagy has in cancer initiation and progression and will describe how autophagy is regulated by important cancer networks. We will also discuss current strategies that are being formulated and tested to target autophagy for cancer therapy.

Regulation of autophagy

Autophagy is an evolutionarily conserved process. It was first genetically defined in yeast, where 31 genes, referred to as autophagy-related genes (*ATG*), have been identified as being directly involved in the execution of autophagy (Mizushima, 2007; Xie and Klionsky, 2007). Many members of this group of genes are well conserved in mammalian cells and, so far, 16 Atg orthologues have been identified in humans.

The initiation of autophagy occurs with the formation of a membranous structure referred to as phagophore or isolation membrane (Fig. 1) (Tooze and Yoshimori, 2010). This membrane is believed to arise from multiple sources within the cell and its formation is initially controlled by a complex containing the two serine/threonine kinases, ULK1 and ULK2, which are orthologues of yeast Atg1, ATG13 and FIP200 (also known as RB1-inducible coiled-coil protein 1, RB1CC1), which is the mammalian orthologue of Atg17 (Axe et al., 2008; Hailey et al., 2010; Ravikumar et al., 2010). The activity of this complex is controlled by the nutrient-sensing kinase mammalian target of rapamycin (mTOR) (Ganley et al., 2009; Hosokawa et al., 2009; Jung et al., 2009). In addition, the nucleation of the phagophore is crucially dependent on the production of phosphatidylinositol 3-phosphate [PtdIns(3)*P*]. The conversion of phosphatidylinositol (PtdIns) to PtdIns(3)*P* is driven by a lipid kinase complex containing human VPS34 [also known as phosphatidylinositol 3-kinase catalytic subunit type 3 (PIK3C3)], vacuolar protein

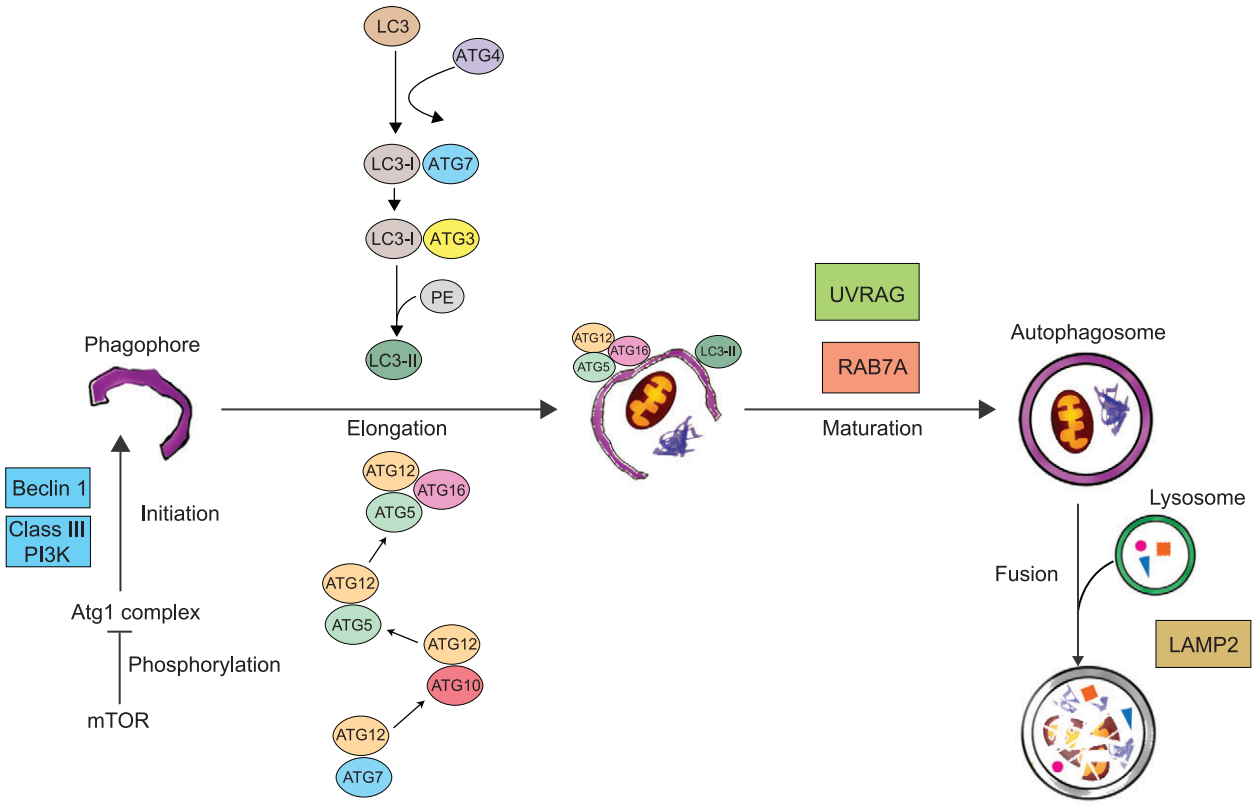

Fig. 1. The pathways controlling autophagy. mTOR is a master regulator of autophagy: it is a bioenergetic sensor and inhibits the ATG1 protein complex by phosphorylating it. In the initiation phase, the Beclin-1–VPS34 complex converts PtdIns into PtdIns(3)*P*, which then recruits two separate ubiquitin-like conjugation systems, resulting in the formation of the ATG12–ATG5–ATG16L and LC3–PtdEtn complexes. This process results in the formation of a vesicle surrounded by a double membrane – referred to as an autophagosome – which engulfs cytosolic contents, such as proteins and organelles. Autophagosomes ultimately fuse with lysosomes to form new organelles, termed autolysosomes, within which the cargo of the autophagosome is degraded by the acidic hydrolases provided by the lysosome. Maturation of autophagosomes and fusion with lysosomes is mediated by UVRAG, RAB7A and LAMP2.

sorting 15 homologue (VPS15) and Beclin 1 (BECN1; the mammalian orthologue of yeast Atg6) (Funderburk et al., 2010). The Beclin 1 core complex can recruit various different proteins to form protein complexes that regulate autophagy in different ways (He and Levine, 2010). For example, Beclin 1 forms a complex with UVRAG (for UV irradiation resistance-associated gene) and Bif-1 [also known as SH3-domain GRB2-like endophilin B1 (SH3GLB1)], to facilitate the curvature of the autophagosome (Liang et al., 2006; Takahashi et al., 2007). By contrast, a complex formed between Beclin 1, UVRAG and a protein called Rubicon results in a complex that inhibits autophagosome maturation (Zhong et al., 2009).

Following the initiation of phagophore formation, the double-membrane grows to enclose cellular contents in a process referred to as the elongation stage of autophagy. PtdIns(3)*P*-containing membranes recruit two ubiquitin-like protein conjugation systems, which are crucial for autophagosome formation (Fig. 1) (Fujita et al., 2008; Hanada et al., 2007; Sou et al., 2008; Xie and Klionsky, 2007). They lead to the formation of the Atg12–Atg5–Atg16L complex and to the conjugation of the protein LC3 (microtubule-associated protein light chain 3) to phosphatidylethanolamine (PtdEtn) (Fig. 1).

Membrane trafficking proteins, including lysosomal-associated membrane protein 2 (LAMP2) and the small GTPase RAB7A, are essential for the docking and fusion of autophagosomes with lysosomes to form autolysosomes (Jäger et al., 2004). Besides its role in autophagosome initiation, UVRAG also controls autophagosome maturation by activating RAB7A (Liang et al., 2008). mTOR also has an additional role at the turnover stage of autophagy. Protein degradation in autolysosomes results in the generation of free amino acids following protein degradation. These amino acids are transferred back to the cytoplasm, leading to local activation of mTOR (Yu et al., 2010), which results in the inhibition of autophagy at these sites and the formation of LC3-negative proto-lysosomal structures from autolysosomes. These extensions finally detach from the autolysome and mature into functional lysosomes, thereby completing a process that has been termed autophagic lysosome regeneration (Yu et al., 2010).

Autophagy and cancer

The initial connection between autophagy and cancer came from two principal lines of evidence. First, it was observed that *BECN1* is monallelically deleted in around 50% of breast, ovarian and prostate cancers (Aita et al., 1999; Liang et al., 1999). Subsequent studies with mice hemizygous for *Becn1* revealed that these mice are viable, but that they display an increased incidence of lymphomas, liver and lung cancers (Qu et al., 2003; Yue et al., 2003). There is now increasing evidence that autophagy has complex and paradoxical roles in tumorigenesis, tumour progression and cancer therapeutics.

Autophagy can function to promote tumour cell survival (Degenhardt et al., 2006) but can also contribute to cell death (Rosenfeldt and Ryan, 2011). It can be upregulated or suppressed by cancer therapeutics, and upregulation of autophagy in cancer therapies can be either pro-survival or pro-death for tumour cells (Levy and Thorburn, 2011; Wilkinson and Ryan, 2010). The exact role autophagy has in cancer is therefore dependent on the context, and we discuss in the following sections the ways in which autophagy can be both tumorigenic and tumour suppressive.

Autophagy as a tumour suppressor

Autophagy is an important mechanism that cells utilise to maintain cellular integrity and genomic stability (Ryan, 2011). Loss of autophagy genes would naturally perturb this homeostasis, thereby potentially priming the cell for tumour development. In this regard, it is important to note that, in addition to the report that *BECN1* hemizygosity is associated with human cancers, tumour-associated deletions or mutations have been found in a number of other autophagy regulators (Table 1). Frameshift mutations in *ATG2B*, *ATG5*, *ATG9B* and *ATG12* have been reported in gastric and colorectal cancers. (Kang et al., 2009). Bif-1, as outlined above, is an autophagy effector involved in autophagosome formation. Its expression is downregulated in gastric and prostate cancers, and mice lacking Bif-1 are prone to tumorigenesis (Takahashi et al., 2007). Monoallelic mutations of *UVRAG* have also been found in a third of colon cancers (Knævelsrud et al., 2010), although autophagy does not seem to be inhibited by the expression of mutant UVRAG or depletion of wild-type UVRAG in cell lines, indicating that this effect on tumour development might occur through an autophagy-independent mechanism (Knævelsrud et al., 2010) (Box 1).

Various mechanisms contribute to the tumour-suppressing effect of autophagy. Under stress conditions, autophagy is activated to remove damaged proteins and organelles, including mitochondria. Inhibition or lack of autophagy results in increased levels of reactive oxygen species and this leads to accumulation of DNA damage, which manifests itself as gene amplification, increased double-strand breaks and polyploid nuclei (Karantza-Wadsworth et al., 2007; Mathew et al., 2007b). This increased DNA damage could lead to a subsequently higher susceptibility to cancer initiation and development.

p62 (also known as sequestosome-1, SQSTM1) is a protein chaperone and signalling scaffold within the cytoplasm (Moscat et al., 2007). p62 protein levels are frequently found to be upregulated in human cancers and this is thought to promote tumorigenesis (Moscat and Diaz-Meco, 2009). Accumulation of p62 leads to increased endoplasmic reticulum (ER) stress and DNA damage (Moscat and Diaz-Meco, 2009) and also contributes to the deregulation of the nuclear factor kappa B (NF-κB) and antioxidant nuclear factor erythroid 2-related factor 2 (NRF2, also known as NFE2L2) pathways in cancer (Duran et al., 2008; Inami et al., 2011; Jain et al., 2010). In the context of autophagy, p62 acts as an adaptor protein that links LC3 with ubiquitin moieties on misfolded proteins. Autophagy therefore mediates the clearance of p62 together with ubiquitylated proteins. Suppression of autophagy hence results in p62 accumulation and contributes to oncogenesis (Moscat and Diaz-Meco, 2009). In this regard, note that in mouse models with either a mosaic deletion of *Atg5* or a liver-targeted deletion of *Atg7*, the benign tumours formed in these animals show an accumulation of p62 (Takamura et al., 2011). Furthermore, deletion of p62 in these mice suppresses tumour growth, which indicates a causative link between p62 accumulation and adenoma formation (Takamura et al., 2011).

Before finishing up the discussion of the role that autophagy has in tumour suppression, it is important to mention that activation of oncogenic signals induces autophagy and this has a role in the establishment of oncogene-induced senescence (Young et al., 2009). Because senescence is considered an important barrier to tumour development in many cancers (Krizhanovsky et al., 2008), this might be another process in which autophagy is required for tumour suppression.

Autophagy as an oncogenic process

There are both mechanistic and genetic studies supporting the hypothesis that autophagy is an oncogenic process. Autophagy is activated as an adaptive mechanism when the intra- and extracellular environment is poor and when cells are metabolically stressed. During the initial stage of tumour formation, cancer cells frequently experience hypoxia and an environment in which nutrients are limited as a result of the tumour growing in the absence of an efficient blood supply (Harris, 2002). These conditions result in metabolic stress and lead to decreased mitochondrial oxidative phosphorylation (Brahimi-Horn et al., 2011). Subsequently, cancer cell proliferation is limited and the cells can enter a dormant state. During the dormancy period, tumour cells rely on autophagy as a survival strategy, whereby nutrients are scavenged to promote cell survival (Lu et al., 2008). Cancer cells can resume proliferation when the stressful environment has improved (Mathew et al., 2007a). Other studies suggest that autophagy is also required for cancer cell survival in more-established tumours. The level of autophagy is elevated in many solid tumours, especially in the less-perfused

Table 1. Autophagy effector genes are frequently mutated in human cancer

Gene name(s)	Stage of autophagy	Type of human cancers	Type of mutation in cancer	References
BECN1	Initiation	Breast, ovarian and prostate cancer	Monoallelic deficiency	(Aita et al., 1999)
UVRAG	Initiation	Colorectal and gastric cancer	Monoallelic deficiency as a result of frameshift mutation	(Ionov et al., 2004; Goi et al., 2003; Kim et al., 2008)
SH3GLB1 (Bif-1)	Initiation	Gastric and prostate cancer	Decreased expression	(Takahashi et al., 2007)
ATG2B, *ATG5*, *ATG9B* and *ATG12*	Elongation	Gastric and colorectal cancer	Frameshift mutation	(Kang et al., 2009)
RAB7A	Fusion	Leukaemia	Gene rearrangement and deletion	(Kashuba et al., 1997)

Box 1. Are the effects of autophagy regulators on cancer related to autophagy?

A number of reports have indicated that perturbations in autophagy regulators can have an impact on tumour development. In addition, many autophagy regulators have been shown to be mutated or silenced in human tumour-derived material. In many cases, however, the autophagy regulator in question has additional cellular roles beyond its connection to autophagy. As a result, it could be the case that the effects these factors have on tumour development are only partly or not at all connected to the role they have in the regulation of autophagy. For example, the p53 target genes *DRAM1* and *DAPK1* have also been shown to be positive regulators of cell death, which, in turn, is a central tumour suppressive mechanism (see Ryan, 2011). FIP200 has also been shown to regulate cell proliferation and Beclin 1 and UVRAG are also known to control endocytosis (Chano et al., 2010; Liang et al., 2008; Ochi et al., 2011; Zeng et al., 2006). Therefore, until a dissection of these different roles is achieved, caution is advised in definitively using these observations to draw conclusions about the role of autophagy in cancer.

areas that contain limited nutrients and oxygen (Mathew et al., 2009a). Moreover, cancer cells with a defect in apoptosis evade necrosis by promoting autophagy as a survival mechanism (Mathew et al., 2007a). In these situations, suppression of autophagy promotes necrotic cell death both in vitro and in vivo (Degenhardt et al., 2006).

A recent genetic study in mice also supports the pro-tumorigenic role of autophagy by highlighting that the deletion of FIP200, which is essential for autophagy, has inhibitory effects on oncogene-driven mammary tumorigenesis (Wei et al., 2011). It remains possible, however, that this effect occurs through a function of FIP200 that is independent of autophagy (Box 1). In this regard, mice with mosaic deletion of ATG5 or ATG7 (two central autophagy regulators) only form benign lesions in the liver, which do not progress (Takamura et al., 2011). Moreover, no lesions (not even benign lesions) are found in other tissues (Takamura et al., 2011). The fact that these tumours do not develop into adenocarcinoma or metastasise indicates that more developed cancers might require autophagy to progress. In addition, the fact that mosaic loss of ATG5 in other tissues does lead to tumour development points to the fact that loss of the protein has tissue-specific effects. The important question is whether loss of ATG5 or ATG7 blocks tumour formation in tissues containing activated oncogenes and lacking important tumour suppressors, as would be the case in natural human cancers, and this has yet to be tested.

In summary, either overactivation or underactivation of autophagy can contribute to tumorigenesis. Because autophagy has opposing roles in tumorigenesis, it has been referred to as a ‘double-edged sword’. On the basis of current scientific understanding, the most popular hypothesis is that autophagy limits tumour initiation but promotes tumour establishment and progression (Fig. 2) (Koukourakis et al., 2010; Mathew et al., 2009b).

Regulation of autophagy by oncogenes and tumour suppressors

In addition to studies that have analysed the effects of autophagy modulators on tumour development, a number of studies have also focused on the ability of known oncogenes and tumour suppressor genes to affect autophagy (Fig. 3). It is not the aim of this Commentary to produce a completely comprehensive list of all the studies in this area because other excellent reviews are available (Maiuri et al., 2009; Rosenfeldt and Ryan, 2009). However, we would like to focus on two proteins – Ras and p53 – which are frequently mutated in human cancers and, similar to the relationship between autophagy and cancer, these proteins have also been shown to have contrasting roles in the control of autophagy.

p53 and autophagy

p53 is a transcription factor that regulates the expression of a spectrum of genes that contribute to tumour suppression (Vousden and Lane, 2007). Its importance in preventing the development of cancer is exemplified by the fact that p53 is mutated in ~50% of human cancers (Soussi and Lozano, 2005). p53 has been reported to modulate autophagy through both its action in the nucleus and through cytoplasmic effects (Kroemer and Levine, 2008). Basal levels of p53 have been shown to repress autophagy in several organisms and this repression is driven by cytoplasmic p53 (Tasdemir et al., 2008). In contrast to the cytoplasmic role of p53 in directing cell death, however, the ability of p53 to repress autophagy involves localisation of p53 to

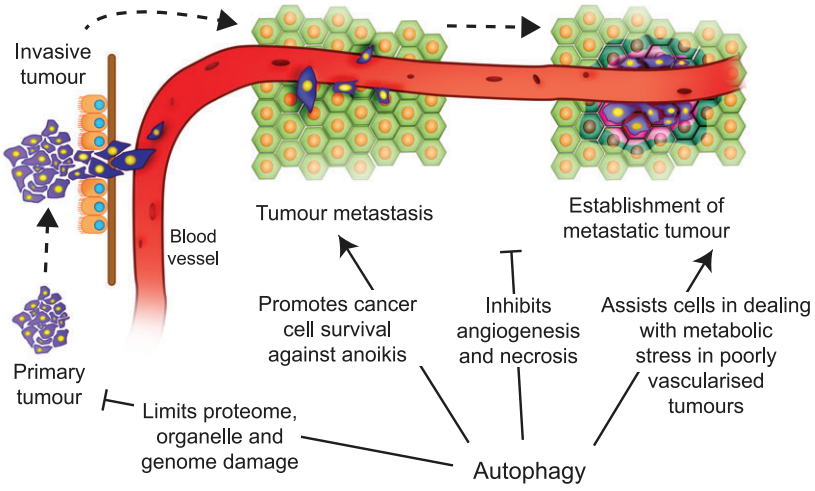

Fig. 2. Autophagy has multiple roles during tumorigenesis. Autophagy can both inhibit and promote cancer formation through different mechanisms, depending on the stage of tumour.

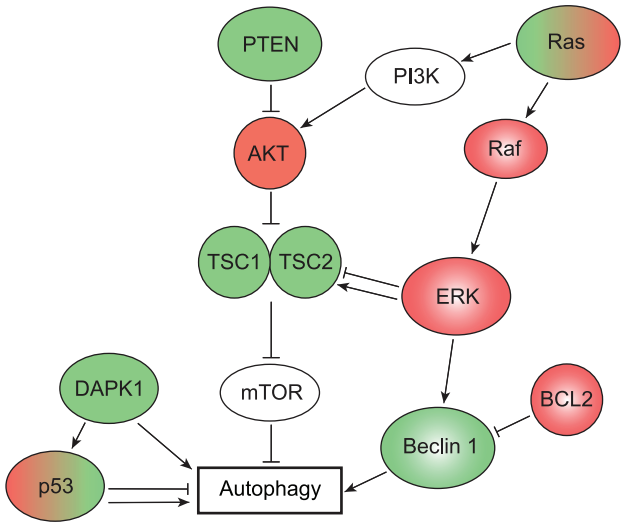

Fig. 3. Oncoproteins and tumour suppressors regulate autophagy. The major cancer networks controlled by p53, Ras and PTEN are regulators of autophagy. In general, oncoproteins act as repressors of autophagy (red) and tumour suppressors act as inducers (green). However, the Ras oncoprotein and the p53 tumour suppressor have been shown to both promote and inhibit autophagy (red and green).

the ER and not to mitochondria (Tasdemir et al., 2008). A recent study suggests that one of the ways cytoplasmic p53 regulates autophagy is through direct interaction with FIP200 (Morselli et al., 2011). In addition, it has been reported that during starvation – which itself is a potent autophagic stimulus – p53 represses activation of the autophagosome membrane protein LC3 through a post-transcriptional mechanism (Scherz-Shouval et al., 2010). This serves to repress autophagy and thereby seems to promote cell survival. At first hand, this seems somewhat paradoxical, because starvation-induced autophagy is widely considered as a pro-survival mechanism. The authors rationalised, however, that p53 does not completely block autophagy by downregulating LC3 but simply prevents excessive autophagy, which could be detrimental to the cell. In support of this hypothesis, the authors showed that knockdown of LC3, and to a lesser extent ATG5, in p53-null cells repressed apoptotic cell death under starvation conditions (Scherz-Shouval et al., 2010).

In contrast to the ability of p53 to repress autophagy, activation and elevation of p53 in response to cellular stress causes the upregulation of a number of genes that promote autophagy (Ryan, 2011). There is a substantial amount of crosstalk between p53 and the mTOR pathway, because p53 can activate genes encoding component AMPKβ1 of AMP-activated protein kinase (AMPK), sestrin 2 (*SESN2*), tuberous sclerosis 1 and 2 (*TSC1* and *TSC2*), and phosphatase and tensin homolog (*PTEN*), which lead to inhibition of mTOR and, by association, to the activation of autophagy (Feng et al., 2007) (Fig. 1). In addition, p53 also regulates other genes that regulate autophagy, most notably damage-regulated autophagy modulator 1 (*DRAM1*). DRAM1 is a lysosomal membrane protein and is a positive regulator of p53-mediated autophagy (Crighton et al., 2006; Crighton et al., 2007b). Interestingly DRAM1 is downregulated in various squamous cancers, indicating that DRAM1-driven autophagy might have a role in tumour suppression (Crighton et al., 2006; Crighton et al., 2007b). The mechanism of how DRAM1 regulates autophagy, however, is still unknown and, because DRAM-1 also contributes to programmed cell death (Crighton et al., 2006), it is possible that a tumour-suppressing effect of DRAM1 is mediated through an autophagy-independent function of this protein (Box 1).

Death-associated protein kinase 1 (*DAPK1*) is another p53 transcriptional target gene. It is a tumour suppressor that promotes autophagy by phosphorylating Beclin 1 and hence relieving its association with B-cell CLL/lymphoma 2 (BCL2) (Zhang et al., 2009; Zalckvar et al., 2009). DAPK1 also inhibits microtubule-associated protein 1B (MAP1B; an LC3-interacting protein that inhibits autophagy). Similar to DRAM1, DAPK1 also has other functions beyond the regulation of autophagy and it is important to consider that additional functions might mediate the effects of DAPK1 with regards to tumour suppression (Martoriati et al., 2005) (Box 1).

In response to genotoxic stress, p53 can also transcriptionally activate *ULK1* and *ULK2*, which are core components of the autophagy machinery. In this context, ULK1 and ULK2 upregulation leads to elevated autophagy, which eventually contributes to cell death (Gao et al., 2011).

p73 is a protein closely related to p53 and the two proteins share many functional characteristics (Dötsch et al., 2010). It was not a surprise, therefore, when p73 was also found to be a modulator of autophagy (Crighton et al., 2007a). Unlike p53, however, although p73 induces DRAM1 expression, its ability to modulate autophagy is independent of DRAM1. This indicates that p73 is able to modulate autophagy through a different target gene or genes. More recent studies have shown that apoptosis enhancing nuclease (*AEN*, also known as ISG20L1) is a target gene of the three p53 family members, p53, p63 and p73 and is a modulator of stress-induced autophagy (Eby et al., 2010). How AEN-induced autophagy is affected by DRAM1 and vice versa in the context of p53-driven autophagy is yet to be determined and is an area worthy of further investigation.

Taken together, the complex functions of p53 in autophagy almost parallel the complex function of autophagy in oncogenesis and tumour suppression. It is clear that the impact of p53 on autophagy is context-dependent and future studies should shed light on whether p53 acts as a pro- or anti-autophagic signal in any given setting.

Ras and autophagy

The Ras family of small GTPases have important regulatory roles in cell growth and cell survival (Schubbert et al., 2007). Recent reports have also connected Ras to autophagy, although whether Ras and its downstream effectors promote or inhibit autophagy depends on the situation (Bodemann et al., 2011; Corcelle et al., 2006; Elgendy et al., 2011; Furuta et al., 2004; Ogier-Denis et al., 2000; Pattingre et al., 2003). Studies have also linked some of the effects of Ras on tumorigenesis to its ability to regulate autophagy. However, as outlined below, depending on context, this link might promote or limit the oncogenic effect of Ras (Elgendy et al., 2011; Furuta et al., 2004; Guo et al., 2011; Yang et al., 2011).

Autophagy has a pro-survival effect when it assists cancer cells to deal with environmental stress, and H-Ras or K-Ras oncoproteins can elevate the levels of autophagy as a pro-survival mechanism (Guo et al., 2011; Lock et al., 2011; Yang et al., 2011). Reduced levels of autophagy in Ras-expressing

cancer cells lead to a failure in the removal of damaged mitochondria, which, in turn, results in impaired oxidative phosphorylation. This, ultimately, impedes the growth of cancer cells containing mutant Ras. Thus, inhibition of autophagy reduces the tumorigenicity of cells expressing mutant Ras (Guo et al., 2011; Yang et al., 2011).

Autophagy is important for maintaining bioenergetics, and is, therefore, also important for tumour cell metabolism. Many cancer cells 're-wire' their metabolic pathways in order to adapt to an altered environment and their rapid growth rate (Deberardinis et al., 2008; Jones and Thompson, 2009). In addition to modulating autophagy, oncogenic Ras can also enhance glycolysis (Hu et al., 2011), a pathway that is heavily utilised by tumour cells to produce energy in a hypoxic environment. The induction of autophagy might be required to maintain energy homeostasis in cells with aberrant Ras, because the inhibition of autophagy in cancer cells expressing mutant Ras decreases their glycolytic capacity (Lock et al., 2011; Kim et al., 2011).

Autophagy also contributes to Ras-mediated resistance to anoikis. Anoikis is a form of apoptosis that takes place when cells are detached from the extracellular matrix (ECM), and it is a protective mechanism against cancer progression (Frisch and Francis, 1994). Tumour cells need to overcome anoikis in order to become invasive and mutant Ras contributes to anoikis resistance through the phosphoinositide 3-kinase (PI3K)–AKT and extracellular-regulated kinase (ERK) signalling pathways (Frisch and Screaton, 2001). A recent study has shown that the induction of autophagy following ECM detachment is a pro-survival mechanism that prevents cells from undergoing anoikis (Fung et al., 2008). There is clinical evidence that hyperactive autophagy in melanoma and hepatocellular carcinoma leads to early metastasis and poor prognosis (Ding et al., 2008; Giatromanolaki et al., 2011).

Paradoxically, in addition to these pro-survival roles for autophagy downstream of Ras in cancer cells, there are also scenarios where Ras-driven autophagy has been shown to be part of a pro-death mechanism. In particular, recent studies have shown that H-Ras-induced autophagy contributes to caspase-independent cell death (Elgendy et al., 2011). Ras upregulates Beclin 1 and knockdown of the key autophagy genes Beclin 1, ATG5 or ATG7 reduces oncogenic Ras-mediated cell death (Byun et al., 2009; Elgendy et al., 2011).

The connection of autophagy to cellular metabolism

Glucose and glutamine are the two most rapidly turned-over nutrients in highly proliferative cancer cells. Besides its above-mentioned role in glycolysis, autophagy is also involved in glutaminolysis, i.e. the catabolism of glutamine. Glutamine breakdown not only promotes cancer cell survival and progression by fuelling the TCA cycle, but also by activating autophagy. In the first step of glutamine metabolism, glutamine is deamidated to produce ammonia. A recent study showed that ammonia diffuses to the outside of the cell during glutaminolysis to act as a signalling molecule that subsequently activates autophagy (Eng et al., 2010). Ammonia–autophagy signalling thus allows the cells that produce ammonia, as well as their neighbours, to deal with this stressful metabolic environment.

Targeting autophagy for cancer therapy

Autophagy is becoming an attractive target for anti-tumour therapies. Autophagy – at least as assessed by the accumulation of autophagosomes – is frequently upregulated in cancer cells following treatment with conventional drugs (e.g. temozolomide), treatment with novel targeted cancer therapies (e.g. tamoxifen) or exposure to ionising radiation (Table 2), such that combination therapies involving autophagy modulators are currently being considered. The role that autophagy has during tumour therapy is, however, complex. Similar to its two-sided effects on tumour development, autophagy can have pro-death or pro-survival roles during cancer therapy (Levy and Thorburn, 2011).

Table 2. Effect of various cancer treatments on autophagy

Name of treatment	Mechanism	Effect on autophagy	Autophagy function (pro-death or pro-survival for cancer cells)	References
Ionising radiation	Induces DNA damage	Induction	Pro-survival	(Chaachouay et al., 2011).
Tamoxifen	Binds and inhibits oestrogen receptors	Induction	Pro-survival	(Schoenlein et al., 2009)
Camptothecan	Downregulation of topoisomerase I and inhibition of DNA synthesis	Induction	Pro-survival	(Abedin et al., 2007)
5-Fluorouracil	Active metabolites that can inhibit thymidylate synthase and become mis-incorporated into DNA and RNA	Induction	Pro-survival	(Li et al., 2010)
Proteasome inhibitors	Inhibition of proteasome	Induction	Pro-survival	(Zhu et al., 2010)
Anti-HER2 antibodies	Inhibition of HER2 receptor signalling	Induction	Pro-survival	(Vazquez-Martin et al., 2009)
Rapamyin and rapamycin analogues	Inhibitor of mTOR	Induction	Pro-death	(Iwamaru et al., 2007; Konings et al., 2009)
Imatinib	Tyrosine kinase inhibitor	Induction	Pro-death	(Yogalingam and Pendergast, 2008; Basciani et al., 2007)
Bafilomycin	Inhibition of autophagy through blocking fusion of autophagosome and lysosome	Inhibition	Pro-survival	(Kanzawa et al., 2003)
Chloroquine	Inhibition of autophagy through blocking fusion of autophagosome and lysosome	Inhibition	Pro-survival	(Amaravadi et al., 2011)
3-methyladenine (3-MA)	PI3K inhibitor	Inhibition	Pro-survival	(Levy and Thorburn, 2011)

Autophagy maintains cell metabolism through self-digestion, and this limits the potential for a metabolic crisis, which, in turn, can lead to necrosis (Degenhardt et al., 2006). In this context, autophagy is a pro-survival response that is exploited by cancer cells to deal with the cytotoxicity inflicted by anticancer agents. A number of studies have now looked at how autophagy can promote drug resistance in response to specific agents. For example, the induction of autophagy delays cell death induced by the DNA-damaging agent camptothecan (CPT) in breast cancer cells (Abedin et al., 2007) and autophagy also has a cytoprotective role in response to 5-fluorouracil (5-FU) in colon and oesophageal cancer cells (O'Donovan et al., 2011; Li et al., 2010). Novel therapies such as the use of proteasome inhibitors (Zhu et al., 2010), anti-HER2 (also known as ERBB2) antibodies (Vazquez-Martin et al., 2009) and kinase inhibitors (Wu et al., 2010) also induce autophagy and this is believed to reduce drug efficacy. Furthermore, the upregulation of autophagy also assists tumour cells to become resistant to ionising radiation (Chaachouay et al., 2011).

At the forefront of efforts to utilise autophagy inhibitors in combination therapy is the use of the anti-malarial drug hydroxychloroquine (Amaravadi et al., 2011). It is known that hydroxychloroquine disrupts lysosomal functions and inhibits the turnover stage of the autophagic pathway. Preliminary studies have indicated that this might present a viable treatment approach. For example, 5-FU is commonly used to treat colorectal cancer and it has been found that hydroxychloroquine can sensitise human cancer cells to 5-FU (Sasaki et al., 2010). 3-methyladenine (3-MA) – another inhibitor of autophagy – had similar effects in this context (Levy and Thorburn, 2011). Similarly, inhibition of autophagy has also been shown to sensitise lymphoma and glioma cells to cancer therapy by facilitating tumour cell apoptosis and, as a result, tumour regression (Amaravadi et al., 2007; Fan et al., 2010). The main question that is central to the use of hydroxychloroquine, however, is whether its effects are mediated by inhibition of autophagy or through other mechanisms. Hydroxychloroquine ultimately inhibits all lysosomal functions, not just autophagy and it also has other effects within the cell, including immunosuppression. Nonetheless, the fact that hydroxychloroquine is already approved for human use and is known to be well tolerated have pushed it forwards in clinical trials. Whether or not its effects are mediated through the inhibition of autophagy or even if it serves to inhibit autophagy in all therapeutic scenarios is yet to be determined, but answers to these questions will hopefully become clear in the near future.

It should be noted that autophagy does not always have cytoprotective roles in response to cancer therapeutics. Because autophagy is a 'self-cannibalistic' process, excess autophagy can also act as a pro-death mechanism that leads to the destruction of cancer cells. There is evidence that autophagy is required for cell death in cancer cells with defects in apoptosis (Mujumdar and Saluja, 2010; Shimizu et al., 2004; Yu et al., 2004). In this case, the lack of autophagy reduces or abolishes the effects of cancer therapeutic agents. Similarly, the mTOR inhibitor rapamycin, which also acts as an inducer of autophagy, leads to the inhibition of proliferation of malignant glioma cells (Takeuchi et al., 2005). In this context, autophagy is considered a crucial pathway that is necessary for rapamycin to mediate its anticancer activities through a pro-death mechanism (Iwamaru et al., 2007). Rapamycin also sensitises prostate cancer cells lacking PTEN to radiation by activating autophagy (Cao et al., 2006).

Autophagy as a prognostic tool

As mentioned above, autophagy can be both upregulated and inhibited by anticancer agents and autophagy in cancer cells can, theoretically, be either beneficial or detrimental for patients during cancer treatment. As a result, accumulation of autophagosomes in tumour cells has been shown to correlate with clinical outcomes in cancer patients and this has raised the possibility of using autophagy markers as a prognostic tool in cancer treatment (Morselli et al., 2011; Giatromanolaki et al., 2011). For example, melanomas with higher levels of autophagosomes are less likely to respond to temozolomide and sorafenib treatment, which leads to a poorer clinical outcome in patients (Giatromanolaki et al., 2011). However, caution should be taken, because correlative studies of this kind rely on either the absence or presence of accumulated autophagosomes as a readout of autophagy. Because autophagosomes represent a mid-point in the whole autophagic process, accumulation of autophagosomes can occur through enhanced induction of autophagy, but can also arise through inhibition of autophagy at a post-induction step, that is, after formation of autophagosomes. As such, accumulation of autophagosomes can represent the inhibition as well as the induction of autophagy.

Concluding remarks and perspectives

In this Commentary, we have outlined that autophagy seemingly has both oncogenic and tumour suppressive roles during tumour development. This might be different in distinct tumour types, at different stages of tumour development or even within separate regions of the same tumour. Similarly, the role carried out by autophagy in cancer therapy also appears to be dependent on the context and even the analysis of autophagy as a prognostic tool is plagued by potential caveats. How then do we successfully target autophagy for tumour therapy? In addition to the complexities within the tumour, one must remember that autophagy also serves to protect organisms against the development of other diseases, including inflammatory conditions and neurodegeneration. Inhibition of autophagy might, therefore, be useful for tumour therapy, but this therapy might at the same time have detrimental effects on normal tissues.

Perhaps then we should be thinking about the design of tumour-selective modulators of autophagy. In this regard, work from our own laboratory has identified a cellular signalling pathway that is required for hypoxia-induced autophagy in tumour cells, while being dispensable for autophagy induced by other stimuli (Wilkinson et al., 2009; Wilkinson and Ryan, 2009). Because hypoxia is, for the most part, a tumour-associated state, these findings form a paradigm whereby a tumour-specific form of autophagy could be targeted by a drug that, at the same time, has little impact on autophagy in normal tissues (Wilkinson and Ryan, 2009). Targeting the signalling pathways that regulate autophagy in specific contexts, as opposed to broadly targeting the autophagy machinery directly, might be the way forwards in utilising regulators of autophagy as a therapeutic target in both cancer and other diseases. Ultimately, however, despite the issues and complexities relating to autophagy in cancer, it is without question that autophagy has an important role in tumour development. To end, therefore, on a note of optimism, it is now widely considered that autophagy modulators will form part of clinical regimens at some point in the future and additional studies are now required to determine how, where and when these agents should be applied.

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